UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/725,309	11/29/2000	Alok Singh	79,212	8594

7590 07/01/2003

Associate Counsel (Patents), Code 1008.2
Naval Research Laboratory
Washington, DC 20375-5000

EXAMINER
HUTSON, RICHARD G

ART UNIT	PAPER NUMBER
1652	14

DATE MAILED: 07/01/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/725,309	SINGH ET AL.	
	Examiner	Art Unit	
	Richard G Hutson	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE ____ MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *22 April 2003* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *3,4,6-15 and 21-24* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *3,4,7-15,21,23 and 24* is/are rejected.

7)☒ Claim(s) *6,22* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 4/22/2003 has been entered.

Applicants previous amendment of the specification, cancellation of claims 1, 2, 5 and 16-20 and amendment of claims 3, 9 and 21, and the addition of new claims 22-24, Paper No. 11, 3/12/2003, has been entered and is acknowledged.

Claims 3, 4, 6-15 and 21-24 are still at issue and are present for examination.

Applicants' arguments filed on 3/12/2003, Paper No. 11, have been fully considered and are deemed to be persuasive to overcome some of the rejections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

Claim Objections

Claims 6 and 22 are objected to because of the following informalities:

Claims 6 and 22 depend from rejected claims 3 and 9, respectively.

. Appropriate correction is required.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 9-14, 21 and 23 are rejected under 35 U.S.C. 103(a) as being unpatentable over Carlsson et al. (Biotechnology and Bioengineering, Vol 51, pages 221-228, 1996), LeJeune et al. (Biotechnology and Bioengineering Vol 54, No. 2, pages 105-114, April 1997), Qiagen Product Guide (1997, pages 106-110), Lu et al. (Journal of Biological Chemistry, Vol 271, No. 9, pages 5059-5065, March 1996) and Polayes et al. (Life Technologies-FOCUS, Vol 16, page 81-84. July 1994).

Carlsson et al. teach the site-specific immobilization of proteins carrying polyhistidine tails. Specifically, Carlsson et al. teach methods for stabilizing the enzymes, lactate dehydrogenase, β-glucoronidase and galactose dehydrogenase by genetically engineering the enzymes to include a poly-histidine tail and binding said enzymes to Sepharose 6B charged with either Zn^{2+} or Cu^{2+}. Carlsson et al. then measured the activity of the immobilized enzymes vs. soluble enzymes and showed that all of the enzymes were active while immobilized. Carlsson et al. further measured long-term stability of the "immobilized enzymes" and showed that all of the "immobilized

enzymes" displayed enzyme activities in the same range or higher as the soluble enzymes.

LeJeune et al. teach that protein immobilization is a common method of enhancing enzyme stability and in particular LeJeune et al. teach the immobilization of phosphotriesterase, a nerve-agent hydrolyzing enzyme, using Hypolpolyurethane prepolymers via covalent binding between the enzyme and the immobilizing Hypolpolyurethane prepolymer.

The Qiagen Product Guide teaches the QIAexpress Ni-NTA (nickel-nitrilotriacetic acid) protein purification system and methods for using this system comprising genetically engineering the insertion of a cDNA sequence encoding a desired protein (to be purified) sequence into an expression vector pQE, such that it inserts a 6X histidine tag into the protein and then adding said 6X His-tagged protein to a Ni-NTA spin column where the 6X His-tagged protein attaches to a Ni-NTA silica base material that is used to purify the 6X His-tagged protein. While Qiagen does not specifically disclose that the attached protein has catalytic activity "while bound", Qiagen does teach that the 6X-his tag rarely affects protein structure or function and need not be removed from the purified protein.

Lu et al. teaches similar methods, as those taught by the Qiagen Product Guide, of protein purification of *E. coli* thioredoxins, with the following exceptions. Lu et al. rather then add a 6X His sequence to the amino or carboxyl terminus of the protein, mutate selective surface exposed residues to histidine and in addition to nickel-nitrilotriacetic acid (NTA) salts, Lu et al. use copper- and nickel-iminodiacetic acid (IDA)

salts. Lu et al. teach that both the IDA and NTA salts resulted in identical protein affinity results (page 5061, top of column 2).

Polayes et al. teach methods of genetically engineering the incorporation of a polyhistidine sequence at either the amino or carboxyl terminus of a protein for use in purifying the expressed protein. Polayes et al. teach that this 6 histidine sequence has a strong affinity for the Ni^{2+}-nitrilo-triacetic acid resin.

One of ordinary skill in the art at the time of filing would have been motivated to use enzyme immobilization of an enzyme, such as the nerve agent hydrolyzing enzyme phosphotriesterase, as a method of enhancing the stability of the enzyme as taught by LeJeune et al and Carlsson et al. As previously stated, LeJeune et al. teach that protein immobilization is a common method of enhancing enzyme stability and in particular LeJeune et al. teach the immobilization of phosphotriesterase, a nerve-agent hydrolyzing enzyme. LeJeune et al. further teach the basis of the motivation for wanting to stabilize an enzyme such as phosphotriesterase is because of "high protein purification costs and limited catalytic lifetimes" of the enzyme limit the feasibility of many processes suitable for utilizing biocatalysis, hence much research has been directed to the minimization of such drawbacks. Enzyme stabilization being such a means of minimizing the drawbacks of high protein purification costs and limited catalytic lifetimes. Enzyme stabilization being an increase in the catalytic lifetime of the enzyme. One would have been motivated to use the method taught by Qiagen Product Guide of genetically engineering the insertion of a 6X histidine tag into the enzyme and allowing the 6X His-tagged protein to attach to a Ni-NTA silica base material as a

means of immobilization that would result in the stabilization of the enzyme, because the method taught by Qiagen Product Guide involves a method of genetically engineering an enzyme to have a poly-histidine tail in a similar fashion as the method taught by Carlsson et al. and the methods taught by Qiagen are readily available for such protein immobilization. One of ordinary skill in the art would have been motivated to use either IDA or NTA salts in the methods taught by the Qiagen Product Guide because each of the different salts works equally well as taught by Lu et al. The reasonable expectation of success comes from the high degree of knowledge in the field as demonstrated by the results of Carlsson et al., the Qiagen Product Guide and Polayes et al.. who successfully genetically engineered a number of different enzymes to contain a poly-histidine tail and those of Carlsson et al. who teach that of all the enzymes immobilized in such a manner, all of them retained enzymatic activity while bound.

Claims 3, 4, 7, 8, 15 and 24 are rejected under 35 U.S.C. 103(a) as being unpatentable over Carlsson et al. (Biotechnology and Bioengineering, Vol 51, pages 221-228, 1996), LeJeune et al. (Biotechnology and Bioengineering Vol 54, No. 2, pages 105-114, April 1997), Singh (U.S. Patent Number. 5,663,387) and Polayes et al. (Life Technologies-FOCUS, Vol 16, page 81-84. July 1994).

Carlsson et al. teach the site-specific immobilization of proteins carrying polyhistidine tails. Specifically, Carlsson et al. teach methods for stabilizing the enzymes, lactate dehydrogenase, b-glucoronidase and galactose dehydrogenase by

genetically engineering the enzymes to include a poly-histidine tail and binding said enzymes to Sepharose 6B charged with either Zn^{2+} or Cu^{2+}. Carlsson et al. then measured the activity of the « immobilized enzymes vs. soluble enzymes and showed that all of the enzymes were active when immobilized. Carlsson et al. further measured long-term stability of the immobilized enzymes and showed that all of the enzymes displayed enzyme activities in the same range or higher as the soluble enzymes.

LeJeune et al. teach that protein immobilization is a common method of enhancing enzyme stability and in particular LeJeune et al. teach the immobilization of phosphotriesterase, a nerve-agent hydrolyzing enzyme, using Hypolpolyurethane prepolymers via covalent binding between the enzyme and the immobilizing Hypolpolyurethane prepolymer.

Singh teaches that polymerizable phospholipids have been used in the stabilization of molecular assemblies and in the development of strategies to expand the usefulness of the lipid assemblies. Singh teaches that it is desirable to provide a means for transporting enzymes and proteins that are immobilized by non-covalent binding and specifically teach methods of polymerizing an amphiphile containing an iminodiacetic acid with other polymerizable amphiphiles, forming vesicles and binding an enzyme, carbonic anhydrase, to the salt on the outer surface of the vesicles. As an example of an enzyme which could be bound to the outer surface of the taught vesicles, Singh teach the use of an enzyme which contains several exposed histidine residues, for example, carbonic anhydrase II, since it contains six histidine residues, four of them available within a distance of 6A. Singh teach that the binding of carbonic anhydrase

occurs via the non-covalent binding or chelating of the Cu^{+2} ion on the surface of the liposomes and that non-covalent binding is preferable to covalent binding because covalent enzyme binding may lead to an alteration or decrease in the enzymes activity.

Polayes et al. teach methods of genetically engineering the incorporation of a polyhistidine sequence at either the amino or carboxyl terminus of a protein for use in purifying the expressed protein. Polayes et al. teach that this 6 histidine sequence has a strong affinity for the Ni^{2+}-nitrilo-triacetic acid resin.

One of ordinary skill in the art at the time of filing would have been motivated to use enzyme immobilization of an enzyme, such as the nerve agent hydrolyzing enzyme phosphotriesterase, as a method of enhancing the stability of the enzyme as taught by LeJeune et al and Carlsson et al. As previously stated, LeJeune et al. teach that protein immobilization is a common method of enhancing enzyme stability and in particular LeJeune et al. teach the immobilization of phosphotriesterase, a nerve-agent hydrolyzing enzyme. LeJeune et al. further teach the basis of the motivation for wanting to stabilize an enzyme such as phosphotriesterase is because of "high protein purification costs and limited catalytic lifetimes" of the enzyme limit the feasibility of many processes suitable for utilizing biocatalysis, hence much research has been directed to the minimization of such drawbacks. Enzyme stabilization being such a means of minimizing the drawbacks of high protein purification costs and limited catalytic lifetimes. Enzyme stabilization being an increase in the catalytic lifetime of the enzyme. One would have been motivated to use the method taught by Singh et al. as a means of enzyme immobilization, involving the immobilization of the enzyme via a

liposome vesicle, wherein said vesicle comprises an amphiphile containing a iminodiacetic acid with other polymerizable amphiphiles, using non-covalent binding as taught by Singh et al. so that undesirable effects such as alterations or decreases in enzyme activity as a result of covalent binding are avoided. One would have been further motivated to genetically engineer the specific enzyme to be immobilized, such that it comprised a string of exposed histidine residues at either the amino or carboxyl terminus of the protein, as taught by Carlsson et al., that could be used in an analogous manner as used by Singh with the internal histidine residues in carbonic anhydrase II to attach the enzyme to the salt on the exterior of the vesicle taught by Singh. The reasonable expectation of success comes from the high degree of knowledge in the field as demonstrated by the results of Singh et al. who successfully attached enzymatically active carbonic anhydrase II via this mechanism and exposed histidine residues internal to the enzyme, to the outside of liposome vesicles as well as the results of Polayes et al. and Carlsson et al. who teach similar means of binding a genetically engineered enzyme using a heterologous polyhistidine sequence and the Nickel ion. Further, Carlsson et al. showed that a number of different enzymes maintained their activity while immobilized via a polyhistidine tail.

Response to Arguments

In response to the previous rejection applicants have amended claim 3 to include the limitations that the claimed methods include the genetic engineering including "one or more terminal histidine residues" rather then "a stabilizing amino acid substitution"

and "wherein the bound enzyme is catalytically active". Applicants continue to traverse this rejection as applied to the newly amended claims. Applicants continue to traverse the above rejection on the basis that the examiner has not cited "specific hint or suggestion" found in one reference that would lead one of ordinary skill in the art to select another reference and combine them, but rather applicants submit that the motivation is found in the present application. This argument is not found persuasive.

As previously stated, one of ordinary skill in the art at the time of filing would have been motivated to immobilize an enzyme, such as the nerve agent hydrolyzing enzyme phosphotriesterase, as a method of enhancing the stability of the enzyme as taught by LeJeune et al. and Carlsson et al. As previously stated, LeJeune et al. teach that protein immobilization is a common method of enhancing enzyme stability and in particular LeJeune et al. teach the immobilization of phosphotriesterase, a nerve-agent hydrolyzing enzyme. LeJeune et al. further teach the basis of the motivation for wanting to stabilize an enzyme such as phosphotriesterase is because of "high protein purification costs and limited catalytic lifetimes limit the feasibility of many processes suitable for utilizing biocatalysis, hence much research has been directed to the minimization of such drawbacks. Enzyme stabilization being such a means of minimizing the drawbacks of high protein purification costs and limited catalytic lifetimes. Stabilization being an increase in the catalytic lifetime of the enzyme. As Singh et al. teaches that polymerizable phospholipids have been used in the stabilization of molecular assemblies, such as enzymes, and specifically teach methods of polymerizing an amphiphile containing an iminodiacetic acid with other polymerizable

amphiphiles, forming vesicles and binding an enzyme, such as carbonic anhydrase, to the salt on the outer surface of the vesicles, Singh et al. provide a means of such "stabilization" through immobilization of the enzyme via multiple histidine residues and the non-covalent binding or chelating of the Cu^{+2} ion on the surface of a liposome as such a way (non-covalently) to preserve enzymes activity.

Thus one would have been motivated to apply the teachings of Singh et al. as a means of achieving the above motivation to stabilize an enzyme, as discussed above and taught by LeJeune et al. Such methods, as taught by Singh et al., involve the immobilization of the enzyme to a liposome vesicle, wherein said vesicle comprises an amphiphile containing a iminodiacetic acid with other polymerizable amphiphiles, using non-covalent binding so that undesirable effects such as alterations or decreases in enzyme activity as a result of covalent binding are avoided. It is acknowledged that Singh et al. uses as an example an enzyme, carbonic anhydrase II, which has a number of internal histidine residues for use in the binding to the liposome, however, most enzymes do not contain internal histidine residues in such a configuration that they would be available to use to immobilize the enzyme as above. In the case of such enzymes, one would have been motivated to use the methods taught by Polayes et al. as discussed above, which include the genetic engineering of a polyhistidine sequence at either the amino or carboxyl terminus of a protein for use in creating an affinity for the Ni^{2+}-nitrilo-triacetic acid resin. One would have been further motivated to genetically engineer the specific enzyme to be immobilized, such that it comprised a string of exposed histidine residues at either the amino or carboxyl terminus of the protein that

could be used in an analogous manner as used by Singh with the internal histidine residues in carbonic anhydrase II to attach the enzyme to the salt on the exterior of the vesicle taught by Singh. The reasonable expectation of success comes from the high degree of knowledge in the field as demonstrated by the results of Singh et al. who successfully attached enzymatically active carbonic anhydrase II via this mechanism and exposed histidine residues internal to the enzyme, to the outside of liposome vesicles as well as the results of Polayes et al. who teach similar means of binding a genetically engineered enzyme using a heterologous polyhistidine sequence and the Nickel ion and the results of Carlsson et al. who showed that a number of different enzymes maintained their activity while immobilized via a polyhistidine tail.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Richard G Hutson whose telephone number is (703) 308-0066. The examiner can normally be reached on 7:30 am to 4:00 pm, M-F.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapu Achutamurthy can be reached on (703) 308-3804. The fax phone numbers for the organization where this application or proceeding is assigned are (703) 305-3014 for regular communications and (703) 305-3014 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Richard G Hutson, Ph.D.
Primary Examiner
Art Unit 1652

rgh
June 30, 2003